Institute Report No. 314

Mutagenic Potential of 1,2-Bis[4-(N-Pinacoloxymethyl)Pyridinium]Ethane Dichloride Hemihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

Suzanne E. Sebastian, BA, SPC, USA
and
Don W. Korte, Jr., PhD, MAJ, MSC

GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of 1,2-Bis[4-(N-Pinacoloxymethyl)Pyridinium]Ethane Dichloride Hemihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 193)--Sebastian and Korte

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Edwin S. Beatrice 14 Mar 94

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The mutagenic potential of 1,2-BIS[4-N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE, oxime.

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 86002

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SPC, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOP's, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE

INCLUSIVE STUDY DATES: 21 April - 24 May 1986

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (LAIR Code TP62) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; and Ms. Joanne Wong provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86002 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr 27 OCT 88

DON W. KORTE, Jr, PHD / DATE
MAJ, MSC
Study Director

Suzanne E. Sebastian 24 October 88

SUZANNE E. SEBASTIAN, BA / DATE
SPC, USA
Principal Investigator

Conrad Wheeler 27 Oct 88

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 1 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 86002

1. This is to certify that in relation to LAIR GLP Study 86002, the following inspections were made:

15 April 1986	-	Protocol Review
21 May 1986	-	Plate Incorporation (TP62)
17 March 1987	-	Plate Incorporation (TP64)
20 March 1987	-	Plate Counting (TP64)

2. The institute report entitled "Mutagenic Potential of 1,2-Bis [4-(N-Pinacoloxymethyl) Pyridinium] Ethane Dichloride Hemihydrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 193, was audited on 23 April 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test-- Sebastian and Korte

INTRODUCTION

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for treatment of nerve agent poisoning. The Ames Test is one of a series of tests in which these compounds will be evaluated to determine their relative potential for further development.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (1).

This evaluation of 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (2). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (LA1R Code TP62) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE

LAIR Code Number: TP62

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM] ETHANE DICHLORIDE HEMIHYDRATE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP62. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by SRI International characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in glass distilled water. Reagent grade water used in this assay was first passed through a Technic Model 301 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega Pure System glass distillation unit (Corning Glass Works, Corning, NY) (3).

Chemical Preparation

On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in glass distilled water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen.

Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (4).

Test Format

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was evaluated for mutagenic potential according to the revised Ames method (2). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (4).

Toxicity Tests:

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM] ETHANE DICHLORIDE HEMIHYDRATE ranging from 1.6×10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since the highest dose showed a decreased number of macrocolonies (below the spontaneous rate) and an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 1.0 mg/plate.

Mutagenicity Test:

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 (batch R-315) was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (5). Plates were incubated upside down in the dark at 37°C for 72 hours (Maron 1985, personal communication). Plates were prepared in triplicate, and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous

revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (2). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The *Salmonella* strains were verified by a standard battery of tests. The integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in all strains except TA1535, TA1537, and TA1538.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (for all strains except TA102).

Five known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 18C-0378), 2-aminofluorene (lot 021547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 015F-0655), and N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-0342), were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (6), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538 (2,4). A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (2) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

A 72-hour rather than a 48-hour incubation period was used. This gave the colonies an additional 24 hours to grow, thus enabling all revertant colonies, especially those of TA102, to be detected with the colony counter.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

On 16 May 1986, the toxicity of 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was determined (Table 1). For this experiment all sterility, strain verification and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed a decrease in the number of macrocolonies and an observable reduction in the density of the background lawn indicating chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 1.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 21-24 May 1986 (Table 2). 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM] ETHANE DICHLORIDE HEMIHYDRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP62

GLP STUDY NUMBER 86002

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION	MEAN	±1SD	BACKGROUND LAWN*
START RUN NEGATIVE CONTROL	77	7.5	NL
5.0 mg/plate	1	1.0	NG
1.0 mg/plate	49	9.6	ST
0.2 mg/plate	62	5.9	NL
0.04 mg/plate	74	10.5	NL
0.008 mg/plate	61	22.5	NL
0.0016 mg/plate	74	12.5	NL
END RUN NEGATIVE CONTROL	92	10.1	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF TP62

GLP STUDY NUMBER 86002

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)†

COMPOUND*	DOSE	TA97		TA98		TA100		TA102	
				WITHOUT S-9					
NEG CONTROL	0.0 mg	94	±10.4	21	±3.0	105	±10.9	168	±7.4
MITO-C	0.5 µg			364	±64.0				
MNNG	2.0 µg	316	±27.7	377	±107.1	310	±74.2		
TP62	1.0 mg	72	±1.5	15	±1.5	112	±7.6	161	±2.6
TP62	0.2 mg	78	±6.6	18	±3.6	117	±2.9	145	±6.5
TP62	0.04 mg	70	±5.8	21	±5.3	109	±6.0	122	±16.6
TP62	0.008 mg	86	±3.2	13	±4.0	112	±10.8	146	±11.2
TP62	0.0016 mg	71	±7.2	17	±1.7	106	±12.5	133	±21.7
TP62	0.00032 mg	78	±14.7	17	±2.1	102	±8.9	131	±11.5
				WITH S-9					
NEG CONTROL	0.0 mg	102	±11.7	26	±3.5	92	±10.6	181	±10.7
AA	2.0 µg			1448	±118.6	1378	±148.6		
AF	2.0 µg	496	±127.1	986	±32.7	437	±80.1	217	±32.1
BP	2.0 µg			281	±92.6	554	±90.3		
TP62	1.0 mg	73	±15.0	22	±2.5	92	±16.4	171	±2.0
TP62	0.2 mg	53	±3.6	23	±2.5	90	±6.0	167	±3.8
TP62	0.04 mg	65	±11.9	25	±2.9	88	±5.7	161	±8.3
TP62	0.008 mg	69	±6.0	23	±2.1	88	±3.5	171	±2.0
TP62	0.0016 mg	80	±11.5	20	±4.2	87	±6.1	162	±7.1
TP62	0.00032 mg	80	±8.3	19	±4.2	80	±1.5	150	±13.5

†Values represent the mean number of revertants/plate (± standard deviation)
*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)†

COMPOUND*	DOSE/PLATE	TA1535		TA1537		TA1538	
		WITHOUT S-9					
NEG CONTROL	0.0 mg	26	±2.6	9	±2.0	23	±2.0
MNNG	20.0 µg	1844	±693.5				
TP62	1.0 mg	20	±1.5	8	±2.0	17	±0.0
TP62	0.2 mg	24	±5.3	6	±2.1	18	±3.8
TP62	0.04 mg	22	±2.6	5	±2.0	13	±3.5
TP62	0.008 mg	23	±4.2	6	±1.5	16	±2.9
TP62	0.0016 mg	21	±3.6	10	±1.5	13	±3.5
TP62	0.00032 mg	15	±3.0	6	±3.8	12	±5.5
		WITH S-9					
NEG CONTROL	0.0 mg	23	±4.4	9	±2.0	22	±2.2
AA	2.0 µg			216	±69.3	1166	±103.7
AF	2.0 µg					420	±61.6
BP	2.0 µg			65	±12.1	101	±4.0
TP62	1.0 mg	15	±4.6	6	±1.0	21	±5.7
TP62	0.2 mg	14	±2.3	8	±3.1	16	±3.5
TP62	0.04 mg	14	±2.6	8	±2.5	17	±2.9
TP62	0.008 mg	11	±2.0	8	±2.5	15	±2.1
TP62	0.0016 mg	15	±3.2	6	±1.0	19	±2.3
TP62	0.00032 mg	13	±3.2	4	±3.6	14	±2.6

†Values represent the mean number of revertants/plate (± standard deviation)
*MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2- aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

A tabular presentation of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, 1,2-BIS[4-(N-PINACOLOXYMETHYL) PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA97, TA98, TA100, TA102) (1,6) or three times (TA1535, TA1537, TA1538) (2,4) the spontaneous revertant colony count. 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that 1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE is not mutagenic when evaluated in the Ames test.

CONCLUSION

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

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APPENDICES

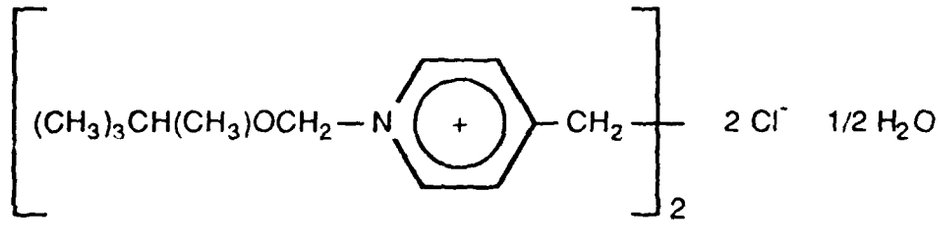
APPENDIX A: Chemical Data

Chemical Name: 1,2-Bis[4-(N-pinacoloxymethyl)pyridinium] ethane dichloride hemihydrate

SRI Reference Number: 6868-16

LAIR Code Number: TP62

Chemical Structure:



Molecular Formula: $C_{26}H_{42}N_2O_2Cl_2 \cdot 1/2\ H_2O$

Molecular Weight: 494.5

Physical State: White crystalline solid

Analytical Data:

NMR (300 MHz, D_2O): δ 0.63 (s, 9 H, $C(CH_3)_3$), 0.96 (d, J = 6.6 Hz, 3 H, $CH(CH_3)$-O), 3.27 (d, J = 6.3 Hz, 1 H, $CH(CH_3)$-O, 3.34 (s, 2 H, N--CH_2), 5.74 (m, J = 5.7 Hz, 2 H, O-CH_2-N), 7.85 (d, J = 7.2 Hz, 2 H, aromatic protons meta to pyridinium nitrogen), 8.75 (d, J = 7.2 Hz, 2 H, aromatic protons ortho to pyridinium nitrogen).* The NMR spectrum obtained upon receipt of the compound corresponded closely to the spectrum provided by the source (obtained in DMSO). Any discrepancies were due to the difference in solvents as well as the higher field strength and greater resolution of the NMR used to analyze the compound in our lab. No peaks other than those attributable to the compound were observed in the NMR spectrum.

Stability:

NMR data demonstrate that the compound is stable in water (D_2O) for at least 8 days.†

Source: Clifford D. Bedford
SRI International
Physical Sciences Division
Menlo Park, CA

*Wheeler CR. Toxicity Testing and Antidotes for Chemical Warfare Agents. Laboratory Notebook #85-12-024, pp 3-4. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX B: Individual Plate Scores

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	2	58	64	85
PLATE 2	0	39	55	64
PLATE 3	1	51	66	74
background lawn	NG*	ST	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG CONTROL START	NEG CONTROL END
PLATE 1	74	65	81	90
PLATE 2	35	88	81	103
PLATE 3	74	68	68	83
background lawn	NL	NL	NL	NL

* NL=Normal Lawn, NG=No Growth, ST=Slight Toxicity

APPENDIX B (cont.): Individual Plate Scores

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
				WITHOUT S-9				
NEG CONTROL (START RUN)	0.0 mg	89	17	113	174	21	9	21
		107	22	118	169	25	9	24
		108	23	103	154	26	12	22
NEG CONTROL (END RUN)	0.0 mg							
		89	21	92	165	28	6	21
		89	17	100	171	28	8	24
		84	24	93	173	27	10	26
				WITH S-9				
NEG CONTROL (START RUN)	0.0 mg	99	32	84	171	24	9	22
		82	24	103	183	25	8	20
		107	27	99	185	19	10	21
NEG CONTROL (END RUN)	0.0 mg							
		117	23	83	164	18	9	23
		107	24	102	191	21	6	25
		100	23	80	189	30	12	19

APPENDIX B (cont.): Individual Plate Scores

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)
POSITIVE CONTROL DATA

COMPOUND*	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
AA	2.0 µg		1577	1432			254	1054
			1344	1210			136	1259
			1422	1492			258	1184
AF	2.0 µg	642	1021	497	196			448
		439	982	468	201			462
		408	956	346	254			349
BP	2.0 µg		210	600			54	100
			248	612			64	105
			386	450			78	97
MITO-C	0.5 µg				392			
					409			
					291			
MNNG	2.0 µg		348	500	267			
			299	327	268			
			301	304	396			
MNNG	20 µg					2134		
						1053		
						2346		

*AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MITO-C=mitomycin C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine

APPENDIX B (cont.): Individual Plate Scores

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP62	1.0 mg	71	15	105	163	20	6	17
		72	13	110	162	22	10	17
		74	16	120	158	19	8	17
TP62	0.2 mg	71	22	115	145	30	8	14
		79	15	120	152	20	7	20
		84	17	115	139	22	4	21
TP62	0.04 mg	67	17	115	138	19	3	13
		67	19	109	124	23	7	10
		77	27	103	105	24	5	17
TP62	0.008 mg	82	18	100	156	20	5	18
		88	11	117	149	22	6	18
		87	11	120	134	28	8	13
TP62	0.0016 mg	65	16	106	149	18	8	17
		79	16	118	141	20	10	11
		69	19	93	108	25	11	11
TP62	0.00032 mg	61	19	95	131	12	3	18
		86	18	99	120	15	4	7
		87	15	112	143	18	10	12

APPENDIX B (cont.): Individual Plate Scores

1,2-BIS[4-(N-PINACOLOXYMETHYL)PYRIDINIUM]ETHANE DICHLORIDE HEMIHYDRATE (TP62)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP62	1.0 mg	57	25	86	171	19	6	27
		74	20	111	173	10	5	16
		87	22	80	169	16	7	19
TP62	0.2 mg	57	25	91	164	13	11	16
		52	23	96	171	13	7	13
		50	20	84	165	17	5	20
TP62	0.04 mg	52	27	86	164	17	10	15
		75	27	94	168	13	5	20
		69	22	83	152	12	8	15
TP62	0.008 mg	69	25	86	169	11	11	13
		75	24	86	171	13	6	17
		63	21	92	173	9	8	14
TP62	0.0016 mg	79	15	90	168	16	5	18
		92	23	91	163	11	6	18
		69	21	80	154	17	7	22
TP62	0.00032 mg	89	24	79	154	12	1	12
		77	16	82	161	11	8	17
		73	18	80	135	17	3	13

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OFFICE OF NAVAL RESEARCH
800 North Quincy Street
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